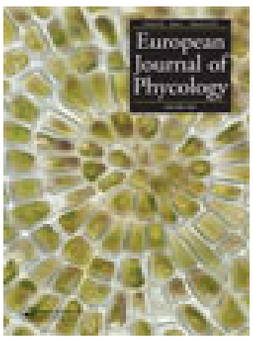

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A rosette by any other name: species diversity in the Bangiales (Rhodophyta) along the South African coast

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ABSTRACT

The Bangiales is an order of Rhodophyta, widely distributed around the globe and best known for its economic value in the nori industry. The morphological simplicity of the group offers limited distinguishing characters for species identification. We therefore delimited species of the Bangiales along the South African coast based on two unlinked loci, the mitochondrial *cox*1 gene and the plastid *rbc*L gene, supplemented with additional sequence data from a third gene, the nuclear nSSU. Application of DNA-based species delimitation methods including the Automatic Barcode Gap Discovery (ABGD), General Mixed Yule Coalescent (GYMC) and Poisson Tree Processes (PTP), resulted in the recognition of 10 *Porphyra* and three *Pyropia* species in South Africa, only three of which had been previously described. Additional species of Bangiales previously recorded along the South African coast were added to our final species list despite not being found in the present study, resulting in an estimate of 14–16 Bangiales species occurring along this shoreline. Most of this extensive genetic diversity has been misidentified as the commonly rosette-forming species *P. capensis*. The name *P. capensis* currently refers to a species complex and cannot be attached to any one species with certainty. All species in this complex, confirmed using genetic data, are endemic to South Africa. Our results compare well with other Southern Hemisphere countries, such as Chile and New Zealand, where high genetic diversity, species richness and endemicity have also been found.

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KEY WORDS Automatic Barcode Gap Discovery (ABGD); Bangiales; *cox*1; General Mixed Yule Coalescent (GMYC); nSSU; Poisson Tree Processes (PTP); *rbc*L

Introduction

The Bangiales are morphologically simple red algae, widely distributed in the marine environment and to a lesser extent in brackish and fresh water. These algae are found from the tropics to the poles, but more commonly occur in temperate regions (Sutherland *et al.*, 2011).

The order consists of one family, the Bangiaceae and was traditionally classified into two genera based on morphology, the bladed *Porphyra* and the filamentous *Bangia* (Engler, 1892; Garbary *et al.*, 1980). However, early molecular phylogenetic data revealed that these two genera were polyphyletic (Oliveira *et al.*, 1995; Müller *et al.*, 1998; Broom *et al.*, 1999). A re-examination of the Bangiales based on two molecular markers (the plastid, ribulose 1,5 bisphosphatecarboxylase large subunit (*rbc*L) gene and the nuclear small subunit rRNA (nSSu) gene) applied to 157 taxa sampled worldwide and using type specimens where possible, revealed 15 well-supported clades. These were circumscribed, reinstated or supported as genera: eight foliose, *Boreophyllum, Clymene, Fuscifolium, Lysithea, Miuraea, Porphyra, Pyropia* and *Wildemania* as well as seven filamentous genera, four of which have been named (*Bangia, Dione, Minerva* and *Pseudobangia*) (Müller *et al.*, 2005; Nelson *et al.*, 2005; Sutherland *et al.*, 2011). Consequently, several species of *Porphyra* and *Bangia* were transferred into new or resurrected genera and a number of undescribed species were highlighted (Sutherland *et al.*, 2011). A ninth bladed genus, *Neothemis*, from the Mediterranean Sea was later added to the order (Sánchez *et al.*, 2014, 2015). Molecular-assisted alpha taxonomy from a series of regional studies thereafter resulted in the recognition of many more (predominantly bladed) species (Kucera & Saunders, 2012; Mols-Mortensen *et al.*, 2012; Mateo-Cid *et al.*, 2012; Nelson, 2013; Nelson & D'Archino, 2014; Ramírez *et al.*, 2014; Sánchez *et al.*, 2014; Lindstrom *et al.*, 2015*a*, 2015*b*; Guillemin *et al.*, 2016).

The most routinely applied molecular markers for species delimitation in the bladed Bangiales are the nuclear encoded nSSU gene, the plastid encoded *rbc*L gene, and to a lesser extent the mitochondrial encoded DNA barcoding gene, cytochrome oxidase subunit 1 (*cox*1) (Robba *et al.*, 2006; Brodie *et al.*, 2008; Kucera & Saunders, 2012; Milstein *et al.*, 2012;

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Mols-Mortensen *et al*., 2012). A comparison of markers showed that the mitochondrial encoded *cox*1 performed best at delimiting species while the other markers were more useful for phylogenetic analyses (Kucera & Saunders, 2012; Ramírez *et al*., 2014; Guillemin *et al*., 2016). At present, some drawbacks of using the *cox*1 gene as a routine species-level marker include the deficient database currently available for the bladed Bangiales, introns that hamper amplification, and the potential inability to detect species using this gene due to hybridization or introgression. Introns can increase the size of a targeted amplicon beyond the limits of successful amplification. For this to be resolved, newly designed primers are required that sit upstream of the intron insertion point and therefore amplify a smaller amplicon. Introns are particularly prevalent in *Pyropia* species, but have also been recorded in other bladed Bangiales (Wang *et al*., 2013; Hughey, 2016). Regarding recently diverging groups, another concern is that two species may share the same *cox*1 gene because of introgression or hybridization, such as *P. umbilicalis* Kützing and *P. linearis* Greville (Mols-Mortensen *et al*., 2012).

Three DNA-based species delimitation methods, the Automatic Barcode Gap Discovery (ABGD), General Mixed Yule Coalescent (GYMC) and Poisson Tree Processes (PTP) have been widely applied recently across a diverse range of organismal groups and are also increasingly used in algal studies (Leliaert *et al*., 2009; Payo *et al*., 2013; Vieira *et al*., 2014; Guillemin *et al*., 2016; Jesus *et al*., 2016; Machín-Sánchez *et al*., 2016; Montecinos *et al*., 2016). ABGD uses DNA sequence data to delimit species by calculating the barcode gap from pairwise distances among samples (Puillandre *et al*., 2012). GMYC estimates species boundaries by calculating the shift from inter-specific to intra-specific branching rates in a phylogeny by fitting a general mixed Yule-coalescent (GMYC) model on an ultrametric gene tree (Pons *et al*., 2006). PTP estimates species boundaries by modelling the speciation rate directly from the number of substitutions in a phylogeny (Zhang *et al*., 2013). More recently, these DNA-based species methods were applied for the first time to bladed Bangiales, which often lack apparent morphological characters for identification. The study revealed extensive species diversity and endemicity in Chile (Guillemin *et al*., 2016). DNA-based species delimitation using unlinked loci therefore appears promising in resolving the taxonomy of morphologically plastic or cryptic groups such as the Bangiales.

Three bangialean genera occur along the South African coast: the filamentous *Bangia sensu lato* (used hereafter) and the bladed *Porphyra* and *Pyropia*. *Porphyra* occurs from Port St. Johns on the east coast to Port Nolloth on the west coast, spanning a distribution range of ~2000 km of coastline (Isaac, 1957; Graves, 1969; Stegenga *et al*., 1997; Jones *et al*., 2004). Species of *Pyropia* (originally described as *Porphyra* spp.) are only known to occur along the south-west and west coast (Stegenga *et al*., 1997; Jones *et al*., 2004), and *Bangia* has only rarely been observed and collected along the west coast of South Africa (John Bolton personal observation 2016).

Bangiales were first reported from the South African coast by Kützing (1843), who recognized two species of *Porphyra*, a reniform to cordate form (hereafter termed 'rosette') and a linear to lanceolate form (henceforth termed 'lanceolate'), both found on the west coast. The rosette form was named *P. capensis* and the lanceolate form, *P. augustinae* Kützing nom. illeg. (see Griffin *et al*. (1999) for further information regarding the legitimacy of names). Both species were later synonymized by J. Agardh (1883) and the name *P. capensis* was conserved. Thereafter, two additional species based on European names, *Porphyra vulgaris* C. Agardh nom. illeg. and *Porphyra lacinata* var. *capensis* (Kützing) Grunow, were recorded in South Africa (Delf & Michell, 1921). However, reviews by Isaac (1957) and Graves (1969) agreed with Agardh and expressed the opinion that only one morphologically variable species, *Porphyra capensis*, occurred in South Africa. However, since then, one new species was described but not named, *Porphyra sp. indet*. (Stegenga *et al*., 1997), and two new *Porphyra* (now *Pyropia*) species were described and named, *Py. saldanhae* (Stegenga, J.J. Bolton and R.J. Anderson) J.E. Sutherland, and *Py. aeodis* (N.J. Griffin, J.J. Bolton and R.J. Anderson) J.E. Sutherland (Stegenga *et al*., 1997; Griffin *et al*., 1999; Sutherland *et al*., 2011). Additionally, two widely distributed *Porphyra* (now *Pyropia*) species, *Pyropia gardneri* (G.M. Smith and Hollenberg) S.C. Lindstrom, and *Py. suborbiculata* (as *P. carolinensis*) (Kjellman) J.E. Sutherland, H.G. Choi, M.S. Hwang and W.A. Nelson, and a cosmopolitan *Bangia* species, *Bangia* cf. *fuscopurpurea* (Dillwyn) Lygbye (as *B. atropurpurea* (Mertens ex Roth) C. Agardh) were recorded from South Africa (Stegenga *et al*., 1997; Griffin *et al*., 1999; Sutherland *et al*., 2011).

Molecular-aided biodiversity studies on the Bangiales in South Africa are largely lacking and to date only a preliminary biodiversity assessment of the bladed Bangiales has been conducted. The study suggested high phylogenetic diversity in the bladed genera (Jones *et al*., 2004; Sutherland *et al*., 2011). The aim of this study was to further explore the biodiversity of the Bangiales following Jones *et al*. (2004) but based on a more extensive collection throughout the known distribution range of these algae along the South African coast.

Because it is well known that data from unlinked loci can provide more reliable estimates of species

boundaries (Knowles & Carstens, 2007; Leliaert *et al.*, 2014), our study is based on two molecular markers: *cox*1 and *rbc*L, and supplemented with information from a third marker, the nSSU gene. The *cox*1 and *rbc*L genes were sequenced and different algorithmic methods for DNA-based species delimitation (ABGD, GMYC, PTP) applied. Results from these analyses were used to first define initial species hypotheses. Sequences for the nSSU gene were obtained from GenBank and used to generate a mulitgene phylogeny. Additionally, we also assessed gross morphological variation and distribution ranges of species. The species delimited in this study based on DNA-sequence data have to be regarded as hypotheses that should be further tested in future studies using detailed morphological, anatomical, eco-physiological and distributional data.

Materials and methods

Taxon sampling

Collection sites were selected across the known South African distribution range where Bangiales were found: East London (33°27'.12"S, 27°51'.16.52"E) to Port Nolloth (29°14'.29.4"S, 16°54'.1.44"E), which included several sites where bladed Bangiales were abundant, particularly on the Cape Peninsula and south-west coast of South Africa (Fig. 1). A survey of Bangiales beyond its known distribution range in South Africa revealed no new records. Blades were collected during 2014–2016 (Supplementary table S1). For the purposes of this study, sites east of Suiderstrand to East London were denoted as the south coast, sites between and including Suiderstrand to the Cape Peninsula were denoted as the south-west coast, and sites north of and including the Cape Peninsula were denoted as west coast sites (*sensu* Stegenga *et al.*, 1997). Additional material from samples used in Jones *et al.* (2004) was acquired and amplified for the *cox*1 gene. However, with the exception of three *Pyropia* amplicons that were long enough for comparisons, these sequences were half the expected size range (~200–300 bp) and were not included in our analyses.

As many different blade forms as possible were collected from various shore positions and from different substrata from 35 sites. Specimens were pressed and preserved as herbarium vouchers, a section from each specimen was removed for DNA analysis and stored in silica gel, and an additional portion preserved in 5% formalin/seawater for anatomical examination. Selected herbarium specimens are deposited in the Bolus Herbarium (BOL) at the University of Cape Town (UCT), South Africa, and all others at the Seaweed Research Unit, Department of Agriculture, Forestry and Fisheries, Cape Town, South Africa.

DNA isolation, PCR-amplification and sequencing

Genomic DNA was extracted using a modified protocol for the DNeasy® Blood and Tissue or Plant

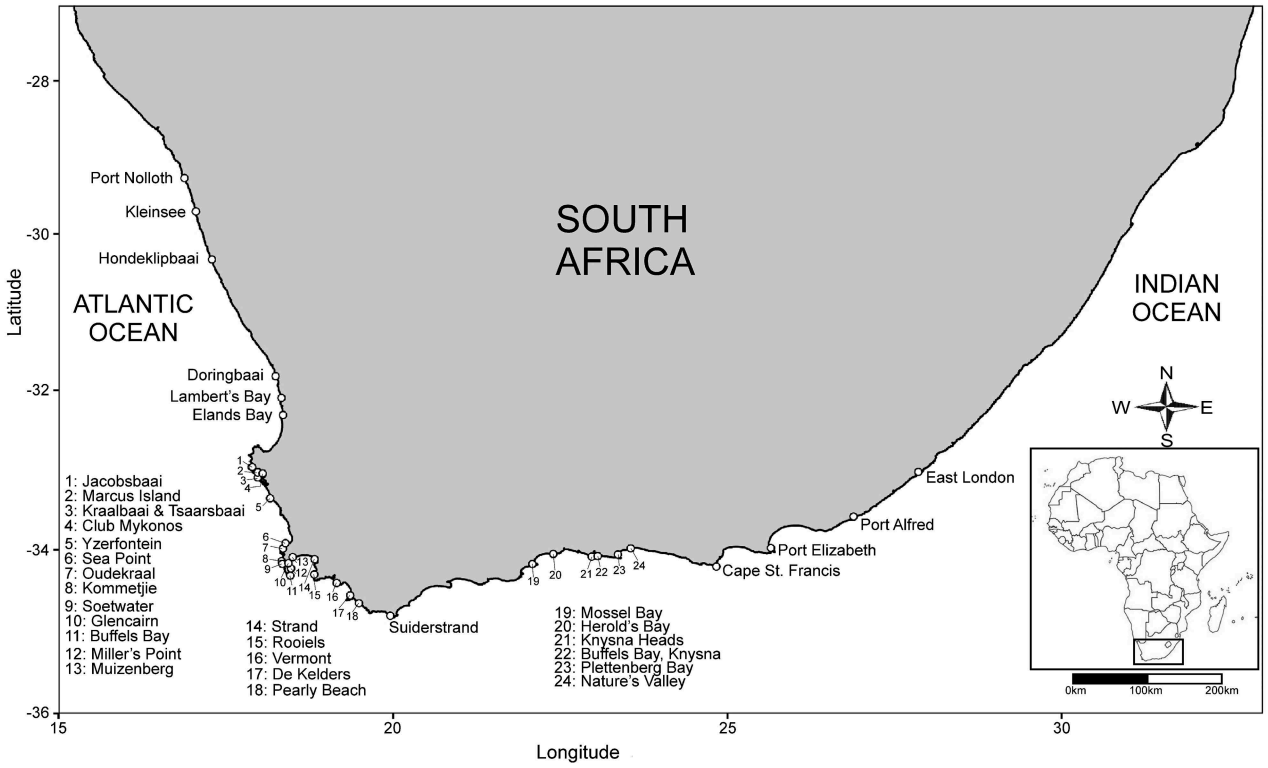

Fig. 1. Collection sites along the South African coastline.

Tissue kits (Qiagen Inc.). Approximately 10–20 mg dried algal material was homogenized in liquid nitrogen using a micropestle in 200 µl microcentrifuge tubes. An initial incubation at 56°C for 45 min followed by 80°C for an additional 15 min ensured higher DNA yields. The quality and quantity of DNA was determined using a Nano-Spec® spectrophotometer. DNA concentrations > 20 µg ml^{-1} were diluted 1:10 using distilled water and concentrations lower than 20 µg ml^{-1} were diluted 1:2.

Two partial gene regions were targeted for PCR-amplification, (1) The plastid, *rbc*L and (2) The mitochondrial, *cox*1 genes using published, adapted or newly designed primers (Broom *et al.*, 2010; Saunders & Moore, 2013; Supplementary table S2). New primers were designed for two known South African species, *Pyropia saldanhae* and *Py. aeodis* and a few *Porphyra* samples that were presumed to contain introns. Primers designed for *Porphyra* specimens were based on an existing *cox*1 dataset. For *Pyropia*, primers were designed based on the species' closest relative (Sutherland *et al.*, 2011; Kucera & Saunders, 2012; Lindstrom & Hughey, 2016) because introns were present in all *Pyropia* specimens (Supplementary table S2).

PCR-reactions for the *rbc*L gene contained a final volume of 25 µl, and the concentration of each component was as follows: 1× PCR buffer, 0.2 mM dNTP of each nucleotide, 1 mM $MgCl_2$, 1.25 mM primers, 1.25 u Taq, 1 ug $µl^{-1}$ BSA, 10–30 ng DNA, the volume was made up to the total by adding PCR-grade water (Qiagen Inc.). PCR-reactions were run on an Applied Biosystems Veritit 96-well thermocycler (Life Technologies, USA) or a Biometra Product Line, Professional Thermocycler (Analytik Jena, Germany). PCR thermo-cycling parameters for the *rbc*L gene followed those of Broom *et al.* (2010) with the exception of the annealing temperature which was set at 50°C. PCR-reactions for the *cox*1 gene were the same as above without additional $MgCl_2$. The optimal temperature profile for the *cox*1 gene used a touchdown PCR protocol, an initial denaturation at 94°C for 5 min, 5 cycles of 94°C for 1 min, annealing at 45°C for 1 min 30 s and an extension at 72°C for 1 min 30 s followed by 94°C for 1 min, annealing at 50°C for 1 min 30 s, an extension at 72°C for 1 min 30 s, and a final extension step at 72°C for 5 min.

PCR products were cleaned using an enzymatic digestion (ExoCIAP) and sequenced at Macrogen (Macrogen Inc., Seoul, South Korea) or the Central Analytical Facilities (Stellenbosch, South Africa) sequence facilities. Sequences were submitted to GenBank under the accession numbers KX852772–KX853026, KY814926–KY814952 and KY799110–KY799111.

DNA sequence datasets

Three datasets were generated: *rbc*L, *cox*1 and a concatenated dataset including nSSU sequences. In addition to the sequences produced during this study, a representative selection of published *cox*1, *rbc*L and nSSU sequences for the Bangiales was added to the dataset (Supplementary table S5). In general, for individual gene trees (*rbc*L, *cox*1) three sequences per species were used except where less than three samples were available (Supplementary table S3; Supplementary figs S1–4). Where several different studies submitted sequences for a single species, one per study was included, therefore *n* per taxon varied from 3–8. A global Bangiales phylogeny following Sutherland *et al.* (2011), but based on a three-gene (*rbc*L, *cox*1 and nSSU) concatenated dataset, and supplemented with new species from updated literature was also reconstructed (Supplementary table S5; Fig. 2; Supplementary fig. S5). DNA sequences were aligned for each gene separately using the Clustal W function in BioEdit (Hall, 1999) and concatenated for the global phylogeny.

Phylogenetic analyses

Each genus was analysed separately, as the inter-generic variation was too high: *Pyropia* species were on average 3× more divergent than *Porphyra* species for the *cox*1 gene (~13%) and 2× more divergent for the *rbc*L gene (~5%). When *Porphyra* and *Pyropia* spp. were initially analysed together, most DNA species delimitation methods failed to detect many known *Porphyra* species as distinct.

The best fitting model for evolution under the Akaike Information Criterion (AIC) was selected for each dataset in Jmodeltest v 2.1.10 (Posada, 2008). For the *cox*1 datasets (*Porphyra*: GTR+I and *Pyropia*: TIM1+I+G) and for the *rbc*L datasets (*Porphyra*: TIM1+I+G and *Pyropia*: GTR+I+G) were implemented in the subsequent phylogenetic analyses. Bayesian inference (BI) and Randomized Accelerated Maximum Likelihood (RAxML) (Stamatakis, 2006) analyses were performed using the programs MrBayes v. 3.2.6 (Ronquist & Huelsenbeck, 2003) and RAxML for web servers (Stamatakis *et al.*, 2008), respectively.

The MrBayes analyses consisted of two independent runs of 5 million generations thinning every 1000 trees using four chains (two hot and two cold) to ensure sufficient mixing. Tree parameters were sampled every 1000 generations and independent runs were viewed in Tracer v. 1.5 (Rambaut & Drummond, 2014) to assess convergence and to determine an appropriate burn-in value which was

Fig. 2. A global phylogenetic gene tree of the Bangiales based on a concatenated dataset (*cox*1, *rbc*L and nSSU genes). South African taxa comprised two genera, highlighted in two subtrees. Support values are indicated at nodes and South African taxa are labelled in red.

set at 25%. Trees were summarized to create consensus trees and calculate posterior probability values. RAxML was run on the web server RAxML Black Box using default parameters and an appropriate evolutionary model according to Jmodeltest. All trees were rooted on their midpoint.

DNA-based species delimitation methods

ABGD analyses were run on the ABGD web server (wwwabi.snv.jussieu.fr/public/abgd) using the default parameters except the Kimura K80 distance model which was implemented over the more simplified

Jukes-Cantor model and the relative gap width (X) varied depending on the dataset (Table 2). Prior to the GMYC and bPTP analyses, sequences were collapsed into unique haplotypes (Supplementary table S4). For the GMYC analysis, an ultrametric tree was constructed in BEAST v. 1.8 (Drummond *et al.*, 2012) using an appropriate model as per Jmodeltest and assuming an uncorrelated lognormal relaxed molecular clock under the constant size coalescent model. Fifty million generations were implemented for two independent runs, sampling every 1000 trees. Runs were inspected for convergence using Tracer v. 1.5 and trees were summarized from the MCMC analyses after discarding the first 25% of trees generated. GMYC analyses were run using a single threshold following Fujisawa & Barraclough (2013) using the SPLITS package in R (R Core Team, 2016). Trees constructed with MrBayes were used as the starting tree for bPTP analyses, which is a Bayesian implementation of the PTP method, run on the web server http://www.exelixis-lab.org/software.html. Singletons refer to a single species, but it is important to note that singletons in haplotypic data may represent several specimens (see Supplementary table S4).

Haplotype networks were constructed for both genera for both genes for which haplogroups and mutations were noted. Pairwise genetic distances (*p*-distances) were calculated in MEGA v. 6.0 (Tamura *et al.*, 2013) implemented for 1000 pseudoreplicates.

Results

A total of 283 sequences (203 *cox*1 and 80 *rbc*L) of South African bladed Bangiales were generated. Sequences for the *cox*1 gene were trimmed to 669 bp, except for a few sequences (those presumed to contain introns) that were shorter in length and trimmed to 350 bp. Intron-containing samples were amplified with alternative primers and therefore produced shorter amplicons. Sequences ranged in size from 864–1409 bp for the *rbc*L gene. South African specimens from this study were resolved in two main clades, corresponding to two genera: *Porphyra* (91% of the *cox*1, and 85% of the *rbc*L sequences) and *Pyropia* (9% of the *cox*1 and 15% of the *rbc*L sequences).

Species delimitation

South African taxa were analysed in the context of already described/named or molecularly identified species obtained from the literature. South African samples, together with GenBank sequences were partitioned into four datasets, one for each genus (*Porphyra* and *Pyropia*) and for each gene (*cox*1 and *rbc*L) (Table 1).

Table 1. Basic phylogenetic information for all samples used in the present study for the two partial genes.

	Porphyra		*Pyropia*	
	*cox*1	*rbc*L	*cox*1	*rbc*L
Total number of sequences/South African (SA) sequences	215/185	133/75	108/18	227/12
Total number of haplotypes/number of SA haplotypes	74/53	98/35	91/15	206/11
Uncorrected p-distance (maximum/average)				
All sequences	0.14/0.04	0.07/0.02	0.19/0.05	0.11/0.04
SA sequences	0.07/0.03	0.04/0.02	0.14/0.06	0.07/0.03

Table 2. ABGD species groups inferred from two partial gene regions for *Porphyra* and *Pyropia*.

	Porphyra		*Pyropia*	
	*cox*1	*rbc*L	*cox*1	*rbc*L
Number of sequences	216	133	108	227
X (relative gap width)	1.0	0.95	1.0	0.95
Prior maximal distance for initial partition	$p = 0.002$	$p = 0.003$	$p = 0.005$	$p = 0.005$
Number of ABGD groups	20	26	41	93
SA[1] ABGD groups	10	7	4	3

[1]SA: South African

ABGD analyses

Twenty ABGD groups were recovered using the *cox*1 gene for *Porphyra*, and South African taxa accounted for half of these (Table 2). The ABGD analysis of the *rbc*L dataset delimited groups that were consistent with the six molecularly identified South African *Porphyra* species according to Jones *et al.* (2004) (ZPP, ZGR, ZBS, ZCE, ZIR, ZDR). An additional molecular species (ZSM) from the coast of South Africa identified by Sutherland *et al.* (2011) was not supported as a distinct species but was instead included in an ABGD group with a number of other species of *Porphyra* such as *P. mumfordii, P. linearis* and a few undescribed species (Supplementary fig. S2). The ABGD analysis recovered 41 *Pyropia* groups using the *cox*1 gene, and 93 ABGD groups using the *rbc*L gene. Two taxa were supported as distinct using both markers (*Py. aeodis, Py. saldanhae*). SW1, 6POR and ZLI were included in a single group using the *rbc*L gene, but the first two were regarded as distinct species using the *cox*1 gene. 1032 was considered distinct from *Py. aeodis* using the *cox*1 gene but included in the *Py. aeodis* group using the *rbc*L gene (Table 2).

GMYC analyses

For the *cox*1 dataset of *Porphyra* the GMYC model was favoured over the null model which is that all sequences belong to a single species ($p < 0.01$). Collectively, 17 clusters and six singletons were

Table 3. *Porphyra* and *Pyropia* species delimited using *cox*1 and *rbc*L gene regions implemented in GMYC using a single threshold.

	Porphyra		*Pyropia*	
	*cox*1	*rbc*L	*cox*1	*rbc*L
Likelihood of null model	538	815	569	1651
Maximum likelihood of GMYC model	543	815	597	1678
Likelihood ratio	11 **	0.14 n.s.	6 ***	574 ***
Number of ML[2] clusters (95% CI)[3]	17 (15–22)	25 (1–26)	26 (24–27)	51 (51–54)
SA[4] ML clusters	10	13	4	3
Number of singletons (95% CI)	6 (5–8)	29 (1–71)	20 (18–21)	54 (45–61)
SA ML singletons	2	9	2	2

[2]ML: Maximum Likelihood; [3]CI: Confidence Interval; [4]SA: South African; ** <0.01; *** <0.001; n.s.: not significant.

identified. South African samples were resolved into 10 of these clusters and two singletons (Table 3). For the *rbc*L dataset of *Porphyra*, the GMYC model was not favoured over the null model ($p = 0.93$); which is reflected by the large confidence interval (95% CI) in the number of Maximum Likelihood (ML) clusters: 1 to 26 species. For the genus *Pyropia*, the GMYC model was favoured over the null model ($p < 0.01$) using the *cox*1 gene. Twenty-six clusters and 20 singletons were identified, of which four clusters and two singletons represented South African taxa (Table 3). Two described South African species (*Py. aeodis* and *Py. saldanhae*) were further split into two and three groups, respectively. Similarly, for the *rbc*L gene, the GMYC model was favoured over the null model. A total of 51 clusters and 54 singletons were delineated. South African taxa were resolved into three clusters and two singletons.

bPTP analyses

A total of 22 *Porphyra* clusters were recovered using the *cox*1 gene; South African taxa accounted for seven clusters and two singletons (Table 4). Using the *rbc*L gene, 49 clusters were identified of which eight clusters and four singletons represented South African taxa (Table 4). For the genus *Pyropia*, using the *cox*1 gene, 44 clusters were recovered and South African taxa accounted for two clusters (*Py. saldanhae* and *Py. aeodis*) and two singletons (Table 4). Using the *rbc*L gene, 111 clusters were delineated, of which three clusters and two singletons consisted of South African specimens.

Table 4. Results of the bPTP analyses based on the *cox*1 and *rbc*L trees for *Porphyra* and *Pyropia*.

	Porphyra		*Pyropia*	
	*cox*1	*rbc*L	*cox*1	*rbc*L
Acceptance rate	0.44	0.47	0.13	0.25
Estimated number of species	11–42	34–69	40–53	99–124
Mean	22	49	44	111
SA[5] ML[6] clusters	7	8	2	3
Singletons	2	4	2	2
SA BI[7] clusters	7	8	2	3
Singletons	2	4	2	2

[5]SA: South African; [6]ML: Maximum Likelihood; [7]BI: Bayesian inference

Final species hypotheses for South African species

The final species delimitation was based on tabulated results of the different species inferred from each of the two loci (Table 5). A 50% majority rule, i.e. when two of the three analytical species delimitation methods (ABGD, GMYC and PTP) were in agreement, was used to decide on consensus species hypotheses following Guillemin *et al.* (2016). More specifically, we recognized species clades that received high clade support in the *cox*1, *rbc*L, and concatenated phylogenies (*cox*1, *rbc*L, nSSU) and were supported by species-level differences in statistical parsimony and genetic distances (Carstens *et al.*, 2013). Additional information on morphology and distribution was taken into consideration when resolving species with unclear boundaries or conflicting results.

In total, 10 species (RSAa-RSAj) for South African *Porphyra* and four species for South African *Pyropia* (RSAk-RSAn) were recognized using the *cox*1 gene. Five were substantiated using the *rbc*L gene: RSAc-d, RSAg, RSAi-j and four supported by the nSSU gene: RSAa, RSAb, RSAi, RSAe. An additional species, ZSM was supported by both *rbc*L and nSSU sequence data. Although, *rbc*L clades were congruent with nSSU clades there were a number of inconsistencies between these clades and the other five *cox*1 species hypotheses (Table 5). The following species hypotheses equate to described species: RSAm = *Pyropia aeodis* and RSAn = *Pyropia saldanhae*. RSAn* = the divergent *Py. saldanhae* clade.

There was generally high consistency among methods using the *cox*1 gene for *Porphyra* except clades RSAa-b were further split in the GMYC analyses (Supplementary fig. S1). In contrast, there was very little congruence between *rbc*L and *cox*1 GMYC clades for South African *Porphyra* (Supplementary figs S1, S2; Table 5). However, in general *Porphyra rbc*L clades compared well with at least half the *cox*1 clades (Supplementary figs S1, S2). On the other hand, all *Pyropia* species hypotheses were generally consistent for both genes and in the concatenated phylogeny, except for RSAl which was consistently recovered as a distinct species using the *cox*1 gene for all methods, but was included in the species *Py. aeodis* using the *rbc*L gene (Supplementary figs S3, S4).

Table 5. Comparisons of methods and markers and final species delimitation.

		Final species delimitation										
Species hypotheses	Entities (Jones *et al.*, 2004)	ABGD *cox*1	GMYC *cox*1	PTP *cox*1	ABGD *rbc*L	GMYC *rbc*L	PTP *rbc*L	Phylogeny nSSU	Concatenated tree	Consensus	Distribution	Morphology
Porphyra RSAaa	New	*	L	*	*	*	*	N/T	*	*	WC	rosette
Porphyra RSAab	ZGR/ZBS	L	*	L	L	*	*	*	*	*	SWC	lanceolate
Porphyra RSAac	ZGR/ZBS	L	L	L	L	*	*	*	*	?	WC	rosette
Porphyra RSAba	ZCE	L	L	L	L	*	*	*	*	?	SWC	rosette and lanceolate
Porphyra RSAb	ZDR	*	S	*	*	S	*	*	*	*	WC & SWC	rosette
Porphyra RSAc	New	*	*	L	L	*	*	N/T	*	*	SWC	rosette and lanceolate
Porphyra RSAd	New	*	*	*	L	*	*	N/T	*	*	SWC	rosette
Porphyra RSAe	ZIR	*	*	*	L	*	*	*	*	*	WC	lanceolate
Porphyra RSAf	ZIR	*	*	*	L	L	L	N/T	*	*	WC	lanceolate
Porphyra RSAg	New	*	*	*	L	*	*	N/T	*	*	WC	lanceolate
Porphyra RSAh	ZIR	*	*	*	L	L	L	N/T	*	*	WC	lanceolate
Porphyra RSAi	ZPP	*	*	*	L	*	*	*	*	*	SC & SWC	rosette
Porphyra RSAj	New	*	*	*	L	*	*	N/T	*	*	SC	rosette
Pyropia RSAk	ZLI	*	*	*	*	*	*	*	*	*	WC	lanceolate to orbicular
Pyropia RSAl	New	*	*	*	L	L	L	N/T	*	*	WC	N/A
Pyropia RSAm	ZAE=*Py. aeodis*	*	*	*	*	*	*	*	*	*	WC	cordiform
Pyropia RSAn	ZEK=*Py. saldanhae*	*	S	*	*	S	*	*	*	*	WC & SWC	lanceolate

'*' denotes congruence; L: lumped; S: Split; WC: West coast; SWC: Southwest coast

Distribution of species of the bladed Bangiales along the South African coast

RSAi was the only strictly south coast species, containing specimens collected from Port Alfred to Mossel Bay. RSAj represented the other south/south-west coast species and included one specimen collected from De Kelders, ~1000 km west of the remaining eight East London specimens included in this cluster. Both species (RSAi and RSAj) did not overlap in distribution with RSAa-h. All other species hypotheses (RSAa-h) occurred sympatrically mostly on the west and south-west coast of South Africa with the exception of five specimens (*Porphyra* sp. CSF 2, KH1, PBB 3,4,6) which were collected on the south coast but were included in one of the west coast species.

Morphological variation in Porphyra *species*

Porphyra species predominantly conformed to one of two morphological forms previously described, rosette or lanceolate (Fig. 3). However, in some cases specimens with a lanceolate form were included in an otherwise predominantly rosette species or vice versa. In some species there was an even split between the number of rosette and lanceolate forms.

Global comparison

Using the *cox*1 gene for *Porphyra*, species groupings were largely consistent with known species using the ABGD, GMYC or PTP methods (Supplementary fig. S2). *Porphyra umbilicalis* and *P. linearis* for the ABGD, GMYC and PTP analyses were recognized as a single species for the *cox*1 gene and all other species groupings were sustained (Supplementary fig. S2). For the *rbc*L gene, results were also largely consistent for known species groupings with some exceptions (Supplementary fig. S3).

For the genus *Pyropia*, when using the *cox*1 gene most known species groupings were sustained (Supplementary fig. S4). For the *rbc*L gene, groupings were consistent for some species, grouped into a single species for others and split into multiple species for some others and a number of mislabelled taxa were evident. For example, *Py. lanceolata* (Setchell and Hus) S.C. Lindstrom was found to appear in more than one species group indicating that the name has been misapplied (Supplementary fig. S5). *Pyropia ishigecola* (Miura) N. Kikuchi and M. Miyata, and *Py. suborbiculata* were considered a single species using the ABGD and PTP methods. These species were retained as mostly separate entities in the GMYC analysis; although the *Py. ishigecola* cluster included a sequence labeled *Py. suborbiculata*.

Genetic distance

Genetic distance matrices were created for each gene (*cox*1 and *rbc*L) and for each genus after checking that names on GenBank were applied correctly at the generic level. A global comparison including known *Porphyra* and *Pyropia* species from the literature was obtained from GenBank and used to calculate intraspecific genetic distances for each genus respectively (Table 6). For both genera and for both genes, intraspecific genetic distances of South African species were within range of published distances (Table 6). Similarly mutational steps, in statistical parsimony,

Fig. 3. Morphological variation in *Porphyra* species along the South African coast. Scale bar represents 25 mm.

Table 6. Mutational steps calculated from statistical parsimony (SP) and pairwise genetic distance comparisons indicating the range of differences among species from around the world, based on *cox*1 and *rbc*L sequence data for *Porphyra* and *Pyropia*. Comparative data for South African taxa are provided.

	Statistical parsimony			
	Porphyra		*Pyropia*	
	*cox*1	*rbc*L	*cox*1	*rbc*L
Global mutational steps in SP (range)	4–8	4–13	3–9	1–6
	Genetic distances (GD)			
	Porphyra		*Pyropia*	
	*cox*1	*rbc*L	*cox*1	*rbc*L
% GD range (average)	1–15 (4)	1–2 (2)	4–21 (13)	1–2 (6)
South African taxa	3–4	1–2	11–14	5–7

between South African taxa compared well with differences found in known species.

Discussion

Species diversity in the bladed Bangiales in South Africa was studied using different methods of DNA-based species delimitation, and this was interpreted in the context of what is known from other *Porphyra* and *Pyropia* species. Extensive species diversity and endemicity was found along this coastline. Intraspecific genetic distances in South African bladed Bangiales were within the range found in currently defined species based on molecular data (Sutherland *et al.*, 2011; Guillemin *et al.*, 2016).

Differences in interspecific genetic distances suggest *Porphyra* is a younger clade with more recently radiating species than *Pyropia*. This may explain the higher consistency in analytical species delimitation methods and congruence in markers for South African *Pyropia* species in comparison to *Porphyra* species.

Species boundaries in the bladed Bangiales from around the globe were largely confirmed in this study, although some species displayed high genetic diversity and may consist of multiple species, as has been found in other species groups (Lindstrom & Cole, 1992; López-Vivas *et al.*, 2015; Lindstrom *et al.*, 2015*a*). In contrast, in some other species, even though morphological and/or ecological species criteria were fulfilled, e.g. *P. umbilicalis* and *P. linearis* or *Py. ishigecola* and *Py. suborbiculata*, genetic diversity among these species pairs was extremely low. These results may reflect hybridization or introgression in these species (Mols-Mortensen *et al.*, 2012). Misapplied names, either taxa that were misidentified or mislabelled, was another concern when estimating taxonomic diversity for global comparisons.

Comparison of molecular markers and species delimitation methods

Recent studies have demonstrated the value of the *cox*1 gene for delimiting species in the Bangiales as it outperforms other gene markers for this purpose, such as *rbc*L and nSSU (Robba *et al.*, 2006; Kucera & Saunders, 2012; Milstein *et al.*, 2012). Although the present study generated information for only two markers, information from a third marker was available from a previous study (Jones *et al.*, 2004) and this allowed for a comparison of genes. Clearer barcoding gaps were obtained using the *cox*1 gene compared with the *rbc*L and nSSU genes and therefore, *cox*1 was the most effective at delimiting species of *Porphyra* and *Pyropia*.

Many recent efforts have focused on adding to the deficient *cox*1 database for the Bangiales (Brodie *et al.*, 2008; Kucera & Saunders, 2012; Mols-Mortensen *et al.*, 2012, 2014; Vergés *et al.*, 2013; Sánchez *et al.*, 2014, 2015; Milstein *et al.*, 2015; Xie *et al.*, 2015; Guillemin *et al.*, 2016). However, introns remain a problem and in the present study two *Pyropia* species and one *Porphyra* species were presumed to contain introns in the *cox*1 region. This required designing new primers for intron-containing species which successfully amplified the *cox*1 gene, but with shorter sequence lengths; nevertheless, these sequences were adequate for comparison.

For the genus *Porphyra*, South African specimens were generally included in the same monophyletic species group using nSSU, *rbc*L or *cox*1, but there was some discordance in gene trees for a few specimens. Phylogenetic relationships between South African *Porphyra* species also varied depending on the marker. This may be a result of recent diversification, incomplete lineage sorting, hybridization or introgression (Mols-Mortensen *et al.*, 2012; Leliaert *et al.*, 2014).

The GMYC method is known to be influenced by completeness of taxon sampling, variability in effective population sizes and the ratio of the effective population size to divergence time, as well as occurrences of rare species (Fujisawa & Barraclough, 2013; Ahrens *et al.*, 2016). The method may also fail to resolve recently diverging taxa in some cases (Hudson & Coyne, 2002; Lohse, 2009; Fujisawa & Barraclough, 2013; Talavera *et al.*, 2013), or conversely, excessively split species (Miralles & Vences, 2013; Ahrens *et al.*, 2016). The excessive splitting in some South African species could, therefore, be accounted for by any of the above mentioned variables.

More specifically, for the genus *Porphyra*, results for GMYC using the *rbc*L gene were not statistically significant and consisted of a large range (1–26 species). A similar trend was observed for other bladed Bangiales studies as well as for other seaweeds (Guillemin *et al.*, 2016; Jesus *et al.*, 2016). Similarly, ABGD groups tended to sort known species into a single species group. Taken together, these results may reflect the absence of a sufficient barcoding gap in this gene, which essentially reduces the taxonomic resolution of species groupings (Meyer & Paulay, 2005; Meier *et al.*, 2008; Kucera & Saunders, 2012). Results from the PTP analysis best reflected species boundaries for *Porphyra* for the *rbc*L gene compared with the other delimiting methods.

On the other hand, analytical methods were congruent using the *cox*1 gene, except for the GMYC results for two *Porphyra* clades and *Pyropia saldanhae* that were further split despite results being statistically significant. These clades consisted of specimens collected from geographically distant sites and the method may be interpreting some level of population structure (Sukumaran & Knowles, 2017). One other consideration is unresolved nodes that may represent real anomalies or methodological artefacts that affect both GMYC and PTP results (Tang *et al.*, 2014). In our dataset this is particularly relevant to the aforementioned *cox*1 clades (see for example *Py. saldanhae*).

Porphyra *species – identifying the elusive* P. capensis

In his initial description, Kützing (1843) referred to *Porphyra capensis* as being rosette in form and the

type locality was listed as Cap which is regarded as *Caput bonae spei* (South Africa). However, in the 1800s this referred to anywhere between modern day Durban and Cape Town. Hypothetically, even if *P. capensis* was considered to be a typical west coast species, it leaves three or four (RSA a–d) possible species that fit the original description. These rosette species typical of the west coast are consistent with entity ZDR and a sequence labelled '*P. capensis*' AY766361 on GenBank (Jones *et al.*, 2004, Milstein & Oliveira, 2005).

Similarly, the identity of the lanceolate form described as *P. augustinae* nom. illeg. (Kützing, 1843) cannot be confirmed at this time, as the description could refer to any one of the lanceolate west coast *Porphyra* spp. or *Pyropia* spp. found in this study. These genera are morphologically similar to one another and can be distinguished largely on reproductive anatomy and to a lesser extent on ecology: South African *Pyropia* spp. are monoecious and found in the subtidal fringe, only occasionally co-occurring with *Porphyra* spp. (personal observation). The original taxonomic sketches by Kützing (1843) provide no information on spore type or arrangement, or details of the ecology or distribution. Nevertheless, one of the lanceolate species, RSAe-h, is confirmed as consistent with the taxon ZIR (Jones *et al.*, 2004). In addition, RSAi recognized in this study is consistent with entity ZPP in Jones *et al.* (2004).

Most other *rbc*L entities, i.e. ZGR, ZBS and ZCE from Jones *et al.* (2004) were either sorted into multiple species or grouped into a single species (RSAa-j), or remained unresolved. An example is the *rbc*L clade ZCE nested in the RSAb clade using the *cox*1 gene.

ZSM (*Porphyra*), a specimen previously collected along the South African coast (Sutherland *et al.*, 2011) was not found during this study but was included in the *rbc*L DNA-based species delimitation analyses. The species was shown to be distinct based on the consensus majority rule and will be included in our final species inventory. Taken together, the name *P. capensis*, therefore, cannot be tied to a single species and at present refers to a species complex until the type specimen is sequenced.

Species boundaries confirmed for two endemic Pyropia *species*

Two endemic '*Porphyra*' species have been described from among the elusive '*P. capensis*', and were later transferred into the resurrected genus *Pyropia* (Sutherland *et al.*, 2011): *Py. aeodis* (Griffin *et al.*, 1999) and *Py. saldanhae* (Stegenga *et al.*, 1997). In the present study the boundaries of both species were confirmed and one new *Pyropia* species as well as a divergent lineage within *Py. saldanhae* has been recognized. The novel species, RSAk shares an almost identical *rbc*L sequence (a single base pair change) with the entity ZLI from a previous study (Jones *et al.*, 2004). All analytical DNA-based species delimitations in the present study identified entity ZLI (Jones *et al.*, 2004) as being conspecific with RSAk (this study). However, this was not reflected in the multigene phylogeny and may be due to the uneven number of gene regions compared between species (ZLI (*rbc*L & nSSU), RSAk (*rbc*L & *cox*1) and the closely related 6POR (*rbc*L & *cox*1)).

A divergent lineage in *Py. saldanhae*, a species that occurs at Rooiels on the eastern shore of False Bay (Fig. 1), was found beyond the known distribution range of this species. Previously documented from the Cape Peninsula to Hondeklipbaai (Stegenga *et al.*, 1997), the divergent lineage appears morphologically distinct, albeit subtly and will require further morphological and anatomical analyses. Although this lineage was genetically distinct, it was insufficiently so to be considered a distinct species, and as such was consistently recognized as belonging to *Py. saldanhae* using tree-based and non-tree-based species delimitation approaches.

A divergent lineage in *Py. aeodis*, RSAl, acquired from an earlier study along the South African coast (Jones *et al.*, 2004) is represented by only a single specimen which was not available for morphological analysis. Furthermore, the length of the *cox*1 sequence for this sample was significantly shorter than other *Py. aeodis* specimens. For the *rbc*L gene, where a more complete sequence was obtained, this taxon was identified as *Py. aeodis*. Therefore, despite all analytical species delimitation methods and genetic distances based on the *cox*1 gene suggesting this may be a new species, we have chosen not to consider it as such until more information is obtained.

The genus *Pyropia* appears to have relatively fewer species and is much less abundant year-round than *Porphyra* in South Africa, so we were only able to sample relatively few *Pyropia* specimens. Intraspecific divergence in South African *Pyropia* species was generally high and it is possible that given a larger dataset, more genetic structure and more species may emerge within this genus.

High diversity and regional endemism hidden under common or misapplied names

For many decades the name *Porphyra capensis* Kützing (1843) was used as an umbrella species to describe what we now know to be two divergent genera (*Porphyra* and *Pyropia*) each consisting of several endemic species (Stegenga *et al.*, 1997; Griffin *et al.*, 1999; Sutherland *et al.*, 2011; this

study). Although these genera are morphologically similar, they are markedly genetically distant (this study; Sutherland *et al*., 2011). Even if we did restrict the name *P. capensis* to include only *Porphyra* species according to the scheme of Sutherland *et al*. (2011), it still conceals extensive species diversity (10 species). These findings are contrary to earlier reviews by Isaac (1957) and Graves (1969) that considered South African bladed Bangiales belonging to a single species.

Similar trends of high diversity and endemism have also been reported for other regions. For example, the name *Porphyra columbina* Montagne (now *Pyropia columbina* (Montagne) W.A. Nelson) and *P. umbilicalis* have been widely applied to species in New Zealand and Chile, and concealed several endemic and new species along both these coastlines (Broom *et al*., 1999; Nelson *et al*., 2001, 2006; Brodie *et al*., 2007, 2008; Nelson, 2013; Nelson & D'Archino, 2014; Ramírez *et al*., 2014; Guillemin *et al*., 2016). Widely applied names in North Atlantic bladed Bangiales were also found to conceal cryptic taxonomic diversity (Kucera & Saunders, 2012; Mols-Mortensen *et al*., 2012, 2014).

Misapplied names and misleading distribution ranges

All South African bladed Bangiales identified molecularly in the current study display regional endemism based on our sampling. However, critical comparisons are needed from subantarctic regions (Gough Island, Tristan da Cunha and Marion Island, where *P. capensis* has been recorded), and from Namibia and southern Angola, where *P. capensis, Py. saldanhae* and *Py. aeodis* have been recorded, based on morphological characters (Papenfuss, 1964; Chamberlain, 1965; Silva *et al*., 1996; Anderson *et al*. 2012; John Bolton and Robert Anderson pers. obs., 2016). Thus, there is a great need for taxonomic clarification of taxa that were previously identified based solely on morphology, particularly with regard to species with a wide range of morphological forms and with wide global distribution ranges (Tronholm *et al*., 2010; Mattio & Payri, 2011; Xie *et al*., 2015).

The widely distributed species, *Pyropia gardneri* which was originally described from California, *Py. suborbiculata* (as *P. carolinensis*) initially described from Japan and *Bangia fuscopurpurea* (as *B. atropurpurea*) which was first described from Germany, were not found in the present study based on DNA sequence data. Furthermore, *Pyropia gardneri* recorded from South Africa (Stegenga *et al*., 1997) morphologically resembles a new endemic South African bladed Bangiales species (RSAk) and requires further study. Therefore, given the difficulty of identifying these species based on morphology (Ramírez *et al*., 2014; Sánchez *et al*., 2014, 2015; Guillemin *et al*., 2016), we suggest that *Py. gardneri* and *Py. suborbiculata* were misidentifications of other species along the South African coast. One other presumed cosmopolitan species, *Bangia* cf. *fuscopurpurea*, was identified based on morphology and recorded along the South African coast. However, no *Bangia* species were found in the present study despite several dedicated seasonal survey trips. Nevertheless, we can conclude with certainty that at least one '*Bangia*' sp. occurs in South Africa but, its identity and endemicity need to be confirmed (Stegenga *et al*., 1997).

The concept of widely distributed macroalgal species has been increasingly challenged in recent times, and many studies reveal regional endemism hidden under widely applied names (Leliaert *et al*., 2009; Payo *et al*., 2013; Vieira *et al*., 2014; Guillemin *et al*., 2016; Jesus *et al*., 2016; Machín-Sánchez *et al*., 2016). In the Bangiales, a few common names, generally for well-studied European species such as *P. umbilicalis* (Brodie *et al*., 2008), have been misapplied to many species from around the world. Similarly, for example, the common European name *P. vulgaris* nom. illeg. has been applied to South African '*Porphyra*' (Delf & Michell, 1921). This is understandable because of a lack of discernible morphological characters in the group, but nevertheless perpetuates the idea of widely distributed bangialean species.

Bangialean species inventory in South Africa

Our analyses suggest that 14–16 species of Bangiales occur along the South African coast, three of which have been previously described and named (*Porphyra capensis, Pyropia saldanhae* and *Py. aeodis*). The name *Porphyra capensis* cannot be reliably assigned to a single species and instead refers to a complex consisting of 10 species. We include the *Porphyra* genetic entity ZMS which was not found in the present study, but for which molecular sequences exist (*rbc*L and nSSU). In addition to two species of *Pyropia* endemic to southern Africa, a new species of *Pyropia* is identified, RSAk. Therefore, in total 14 species are recognized. The final estimate included two additional species that require verification. These were *Bangia* cf. *fuscopurpurea* and *Pyropia* cf. *suborbiculata*, the identity and generic placements of which, however, need to be determined. The endemic, *Porphyra sp. indet*. (Stegenga *et al*., 1997), has not been found again since its description and it is therefore doubtful that this species represents a distinct entity. All three of these species are currently lacking molecular data. For reasons mentioned above, the widely distributed *Py. gardneri* has been tentatively removed from the South African flora until further research is conducted. Earlier taxonomic circumscriptions that

were synonymized with *P. capensis* (*P. augustinae* nom. illeg., *P. vulgaris* nom. illeg. and *P. lacinata* var. *capensis* (Kützing) Grunow) were also excluded from our final inventory.

In conclusion, we found extensive diversity, regional endemism and geographic structure in the Bangiales along the South African coast. Phylogenetic diversity was considered in the context of currently accepted species boundaries, using different DNA-based species delimiting methods and a multigene phylogeny. The relative efficacies of these methods were compared and despite some differences, a high level of congruence was found between molecular markers and methods. Our results demonstrate the value of applying a statistical framework when defining species boundaries in taxonomically challenging groups such as the Bangiales; allowing for reproducibility while minimizing the inherent subjectivity associated with defining species boundaries. Although several established species boundaries from other regions outside South Africa were affirmed, our analyses suggest that a high level of species diversity is waiting to be discovered. In particular, the South African coast proved to be a repository for undiscovered species and although our study was based on an extensive collection throughout its distribution range, species are known to occur seasonally and further sampling may result in the recognition of more species from this coastline. In the present study, species were based on molecular information and these species hypotheses need to be further explored using detailed morphological, anatomical and distributional data. Our findings provide a good indication of the total number of Bangiales in South Africa and largely contribute toward understanding the biodiversity of the Bangiales on a global scale. Furthermore, this study forms the basis for future research on the evolution, ecology and biology of this hyper-diverse species complex in the Southern Hemisphere. Lastly, future work should focus on identifying commercially important species/strains from South Africa.

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Disclosure statement

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Supplementary information

The following supplementary material is accessible via the Supplementary Content tab on the article's online page at https://doi.org/10.1080/09670262.2017.1376256

Supplementary table S1. Sample collection dates and sites.

Supplementary table S2. Information on primers used and designed in this study.

Supplementary table S3. Accession numbers for all specimens acquired from GenBank for the *cox*1 and *rbc*L gene for trees in fig. S1–4.

Supplementary table S4. Haplotype list of sequences used for phylogenetic analyses for both genera, and for both genes.

Supplementary table S5. Accession numbers for all specimens acquired from GenBank for the *cox*1, *rbc*L and nSSU genes for the global phylogeny in figs 2 and S5.

Supplementary fig. S1. Phylogenetic gene tree based on the *cox*1 gene for the genus *Porphyra* including South African sequences generated in this study.

Supplementary fig. S2. Phylogenetic gene tree based on the *rbc*L gene for the genus *Porphyra*.

Supplementary fig. S3. Phylogenetic gene tree based on the *cox*1 gene for the genus *Pyropia*.

Supplementary fig. S4. Phylogenetic gene tree based on the *rbc*L gene for the genus *Pyropia*.

Supplementary fig. S5. Global phylogeny of the Bangiales based on a concatenated dataset (*cox*1, *rbc*L and nSSU).

Author contributions

M.M. Reddy: original concept, field work, lab work, data analyses, drafting and editing manuscript; O. De Clerck: assistance with lab work, data analyses and editing manuscript; F. Leliaert: assistance with data analyses, generating trees and editing manuscript; J.J. Bolton: original concept, assistance with field work, editing manuscript; R.J. Anderson: original concept, assistance with field work; editing manuscript.

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